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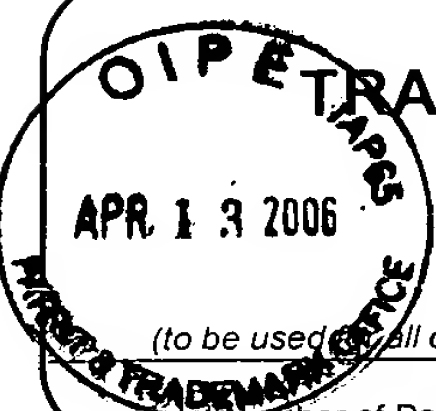
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PTO/SB/21 (09-04)

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	Application Number	09/831,047	Certificate APR 19 2006 of Correction	
	Filing Date	May 3, 2001		
	First Named Inventor	Blaszcyk-Thurin et al		
	Art Unit	1653		
	Examiner Name			
Total Number of Pages in This Submission		45	Attorney Docket Number	WST93AUSA

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment/Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Reply to Missing Parts/Incomplete Application <input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation <input type="checkbox"/> Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____ <input type="checkbox"/> Landscape Table on CD	<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) (please identify below): 3 pp. Request for Certificate of Correction under 35 USC Section 254 2 pp. Forms PTO/SB/44
Remarks 9 pp. Copies of Issued patent pages with errors marked in red 30 pp. Copies of specification pages, correction of filing receipt pages, and Issue fee pages, with words or phrases highlighted in blue. Express Mail No. ED378262197US		

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/831,047 Confirmation No.: 8220
Applicant : Blaszczyk-Thurin et al
Filed : May 3, 2001
Patent No. : 6,960,566
Issued : November 1, 2005
TC/A.U. : 1653
Examiner :
Customer No. : 00270
Title : COMPOSITIONS AND METHODS FOR
TREATMENT OF CANCER

Commissioner for Patents
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Sir:

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 35 USC SECTION 254

The following errors were found in the above-identified patent:

- (1) First page, Cover Page of patent, add inventor "Thomas Kieber-Emmons, Newtown Square, PA" under (75) Inventor;
- (2) First page, Cover page of patent, under Assignees:, replace "Wister" with -- Wistar -- .
- (3) Col. 3, line 1, replace "firther" with -- further -- ;
- (4) Col. 6, line 17, replace "DLWDWWGKPAG" with -- DLWDWVVGKPAG -- ;
- (5) Col. 7, line 31, replace "VGIWSWSEGSR" with -- VGIWSVVSEGSR -- ;
- (6) Col. 7, line 36, replace "LAEMMG" with -- LAEMFMG -- ;
- (7) Col. 17, line 67, replace "3:883" with -- 33:883 -- ;

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APR 20 2006

- (8) Col. 18, line 12, replace "25:1078-1082" with -- 257:1078-1082 -- ;
- (9) Col. 18, line 25, replace "arc" with -- are -- ;
- (10) Col. 24, line 48, replace "carbodiimide" with -- carbodiimide -- ;
- (11) Col. 27, line 10, replace "DLWDEVVGKPAG" with -- DLWDFVVGKPAG -- ;
- (12) Col. 27, line 41, replace "night" with -- might -- ;
- (13) Col. 27, line 50, replace "DLWVDWVVDKPAG" with -- DLWDWVVGKPAG -- ;
- (14) Col. 34, line 66, replace "claims to be" with -- claims are intended to be -- ;
- (15) Col. 69, lines 20 and 21, Claim 1, replace, "GNCRYIGLRQPG" with -- GNCRYIGLRQFG -- ;

It is requested that a Certificate of Correction be issued to correct the above error in accordance with the enclosed Form PTO 1050, which is submitted herewith.

These errors are typographical in nature and make no substantive changes. All errors were made by the USPTO, no fee is due for correction of these errors.

Enclosed for each correction is a photocopy of the original specification page with the relevant words or phrases highlighted in blue and the corresponding original patent with errors marked in red. These documents will support the USPTO errors.

Also enclosed are copies of documents that support the correction of errors (1), (2), and (15); a description of each is listed. A copy of a Request for Correction of Filing Receipt that was filed by first class mail on May, 9, 2002 supports error (1). A copy of an issue fee that was filed by first class mail on January 25, 2005 supports error (2). A copy of a Response and Amendment that was filed by via fax on December 6, 2004 supports error (15).

APR 20 2005

The Director of the US Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,

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UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO : 6,960,566

APPLICATION NO. : 09/831,047

Page 1 of 2

ISSUE DATE : November 1, 2005

INVENTOR(S) : Blaszyk-Thurin et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

- (1) First page, Cover Page of patent, add inventor "Thomas Kieber-Emmons, Newtown Square, PA" under (75) Inventor;
- (2) First page, Cover page of patent, under Assignees:, replace "Wister" with -- Wistar -- .
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- (10) Col. 24, line 48, replace "carbodiimide" with -- carbodiimide -- ;

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UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO : 6,960,566

APPLICATION NO. : 09/831,047

Page 2 of 2

ISSUE DATE : November 1, 2005

INVENTOR(S) : Blaszczyk-Thurin et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

- (11) Col. 27, line 10, replace "DLWDEVVGKPAG" with -- DLWDFVVGKPAG -- ;
- (12) Col. 27, line 41, replace "night" with -- might -- ;
- (13) Col. 27, line 50, replace "DLWVDWVVDKPAG" with -- DLWDWVVGKPAG
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- (15) Col. 69, lines 20 and 21, Claim 1, replace, "GNCRYIGLRQPG" with -- GNCRYIGLRQFG -- ;

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APR 20 2006



(12) **United States Patent**
Blaszczyk-Thurin

(10) **Patent No.:** **US 6,960,566 B1**
(45) **Date of Patent:** **Nov. 1, 2005**

(54) **COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER**

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Philadelphia, PA (US)

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patent is extended or adjusted under 35
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(21) Appl. No.: **09/831,047**

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(2), (4) Date: **May 3, 2001**

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1998.

(51) Int. Cl.⁷ **A61K 38/00; C07K 5/00**

(52) U.S. Cl. **514/14; 530/300**

(58) Field of Search **514/2, 14, 1; 530/300**

(56) **References Cited**

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(Continued)

Primary Examiner—Jon Weber

(74) *Attorney, Agent, or Firm*—Howson and Howson

(57) **ABSTRACT**

Compositions containing one or more peptido-mimetics or modified peptido-mimetics of a carbohydrate ligand of an adhesion molecule in a physiologically acceptable carrier are useful for methods of reducing metastasis in a mammal and for inhibiting inflammatory response in a mammal. Particularly useful are embodiments in which the ligand is a Lewis antigen and/or the adhesion molecule is a selectin, e.g. E-selectin. Methods are disclosed for identifying peptido-mimetics of carbohydrate ligands, which may be involved in binding of tumor cells to other cells, such as endothelial cells.

In vivo studies have provided further evidence of the potential importance of the carbohydrate ligand/E-selectin interaction in tumor metastasis [Brodt et al., 1997, *Int. J. Cancer* 71:612-619; Mannori et al., 1997, *Am. J. Pathol.* 151:233-243 (Mannori II), Biancone et al., 1996, *J. Exp. Med.* 183:581-587].

Alternatively, some carcinoma cells do not express these carbohydrate determinants (i.e., SA-LeX and SA-Le^a) and yet they can attach to EC prior to activation. Further, this adhesion is not augmented by cytokine treatment, suggesting E-selectin-independent adhesion [Iwai et al., 1993, *Int. J. Cancer* 54:972-977, Tozeren et al., 1995, *Int. J. Cancer* 60:426-431; Miyake et al., 1992, *New Eng. J. Med.* 327:14-18; Garrigues et al., 1992, *J. Cell. Biol.* 125:129-142].

Studies have also demonstrated the role of oligosaccharides in inflammatory responses. Neutrophil extravasation is enabled by a multistep process initiated by the selectin family [Kansas, 1996, *Blood* 88: 3259-3287]. Neutrophil-endothelial cell interaction mediated via the selectins in the context of vascular shear flow, are characterized by transient tethering of the neutrophils, followed by rolling of the neutrophil along the endothelial surface of the vessel wall. Studies in vivo and in vitro indicate that selectin-dependent neutrophil rolling is essential to subsequent events in the transmigration process. Neutrophils are exposed to endothelial cell derived IL-8, platelet-activating factor and other neutrophil-activating molecules [Lowe, 1997, In: *The selectins: Inhibitors of leukocyte endothelial adhesion*, pp. 143-177, Vestweber, ed., Harwood Academic Publishers, Reading, UK], which in turn promote activation of neutrophil P2 integrins, leading to integrin-dependent firm adhesion to the integrin receptor ICAM-1, and finally to neutrophil extravasation, possibly via homophilic interaction of platelet/endothelial cell adhesion molecule 1.

The expression of ligands for selectins, particularly E-selectin, by both neutrophils and carcinoma cells raises the possibility that metastases are equivalent to the inflammatory process in which tumor cells, particularly carcinoma cells, use the same molecular mechanism(s) for cancer cell-EC interaction as lymphocytes, through the adhesion interaction of the endothelial cell selectins with the tumor-associated carbohydrate ligands, e.g., SA-LeX, SA-Le^a, and LeY.

In addition to their role in cell adhesion, carbohydrate structures also play a role in angiogenesis. Aberrant angiogenesis can occur in a variety of pathologic conditions. Neovascularization of tumors occurs by aberrant stimulation of normally quiescent endothelial cells to migrate, proliferate and form new capillary blood vessels [Ingber and Folkman, 1989, *J. Cell. Biol.* 109:3317-3330]. The experimental evidence suggests that E-selectin and its ligand SA-LeX function in angiogenesis [Nguyen et al., 1982, *J. Biol. Chem.* 267:26157-26165]. Thus, proliferating microvascular endothelium presents a potential target for anti-cancer and anti-angiogenic therapies through the inhibition of E-selectin-dependent carbohydrate-mediated interactions [Folkman, 1995, *N. Engl. J. Med.* 333:1757-1763].

Although recent studies suggest the importance of carbohydrate ligand-cell adhesion interactions in tumor metastasis, angiogenesis and inflammatory responses, the complex nature of the carbohydrate ligands involved has long hampered studies of these processes. The difficult chemical or enzymatic synthesis required by these complex carbohydrate ligands and the technical complexity involved in analyzing the functional/structural interactions of these

ligands with selectins at the molecular level have severely hindered the development of anti-adhesion therapeutics for treatment of these disease processes for which there is no effective treatment.

Many peptide mimics of carbohydrate structures have been described in the literature [see, e.g., Agadjanyan, M. et al., 1997, *Nature Biotechnol.* 15: 547-551, among others] including those binding with high affinity to E-selectin [Tsukida, T. et al., 1998, *J. Med. Chem.* 41: 4279-4287]. A peptide that mimics the GD1 ganglioside, also involved in cell adhesion and metastasis of melanoma cells, has been recently described [Ishikawa, D. et al., 1998, *FEBS Lett.* 441: 20-24]. This peptide isolated from a peptide phage display library using an anti-GD1 antibody inhibits metastasis in an in vivo model.

Thus, there remains a long-felt and acute need for the development of techniques and probes for the study of complex carbohydrate ligand-cell adhesion molecule interactions, and for the development of anti-tumor, anti-inflammatory and angiogenesis-blocking therapeutics based on the selective inhibition of these interactions.

SUMMARY OF THE INVENTION

The present invention meets the above-stated needs by providing novel peptido-mimetics of carbohydrate structures and uses therefor in affecting animal cell adhesion mediated by carbohydrate ligand-cellular lectin protein receptor interactions.

In one aspect, the invention includes a composition comprising a peptido-mimetic of a carbohydrate ligand of an adhesion molecule in a physiologically acceptable carrier. In one embodiment, the adhesion molecule is a selectin, particularly E-selectin. In another embodiment, the ligand is a Lewis antigen. Particularly desirable are peptidomimetics of the Lewis antigens SA-Le^a, SA-LeX, and LeY. A variety of specific peptido-mimetics are recited in the detailed disclosure such peptido-mimetics may be modified as described herein.

In still another aspect, the invention provides a method of modulating binding of an adhesion molecule to a carbohydrate ligand. The method comprises contacting the adhesion molecule (e.g., a selectin) with a peptido-mimetic of the carbohydrate ligand, so that binding of the adhesion molecule to the carbohydrate ligand is modified or altered in a therapeutically effective manner.

In a further aspect, the invention provides a method of modulating adhesion of a tumor cell to an adhesion molecule located on an endothelial cell. The method comprises contacting the tumor cell with a peptido-mimetic of a carbohydrate ligand. The peptido-mimetic modulates adhesion of the tumor cell to the endothelial cell. The adhesion molecule may be, e.g., a selectin. The ligand is preferably a Lewis antigen.

In still another aspect, the invention provides a method of treating cancer in a mammal by administering an effective amount of a peptido-mimetic of a carbohydrate ligand to the mammal. The ligand is preferably a Lewis antigen. Administration of the peptido-mimetic reduces adhesion of tumor cells to endothelial cells in the mammal, thereby reducing metastasis of the cancer.

In yet a further aspect, the invention provides a method of inhibiting an inflammatory response in a mammal by contacting an endothelial cell with an effective amount of a peptido-mimetic of a carbohydrate ligand. The ligand is preferably a Lewis antigen. The specific peptido-mimetics described herein can be used in this method.

As another aspect, the invention provides additional methods of identifying a variety of peptido-mimetics of carbohydrate ligands. The peptido-mimetics inhibit the normal binding between the ligand and its natural binding partner. In one embodiment, the binding partner is an adhesion molecule, such as a selectin. In another embodiment, carbohydrate ligand is located on the surface of a tumor cell and is a Lewis antigen. In a further embodiment of this method, the carbohydrate ligand is located on the surface of a tumor cell and it normally binds to an adhesion molecule on human umbilical cord vein endothelial cells (HUVEC). In another embodiment, the carbohydrate ligand affects capillary tube formation or angiogenesis. In still another embodiment, the peptido-mimetic affects adhesion of a selected cell, e.g., a neutrophil or tumor cell, to an adhesion molecule located on an endothelial cell. In another embodiment, the carbohydrate ligand affects neutrophil recruitment. The steps of these methods are described in detail below.

In yet another aspect, the invention provides a method of producing peptido-mimetics of Lewis antigens, particularly peptido-mimetics not including APWLYAGP [SEQ ID NO: 83]. This method comprises the steps of screening a random peptide library, in which the peptides are expressed as fusion proteins on the surface of bacterial clones, with antibodies specific for the Lewis antigens and/or an E-selectin immunoglobulin fusion protein; and selecting clones which bind the antibodies and/or the fusion protein. The selected clones produce peptido-mimetics of the Lewis antigens.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a diagram depicting the reduction of neutrophil influx upon administration of DLWDWVVGKPAG [SEQ ID NO: 1] mimicking SA-Le^a carbohydrate in mice with chemically induced peritonitis. The neutrophil count results were obtained from four experiments (three mice in each group). Unrelated peptide was administered in control mice. Statistical analysis of the data using nonparametric unpaired t-test yielded P values <0.001 for the data shown in FIG. 1A.

FIG. 1B is a diagram illustrating the myeloperoxidase activity in the collected neutrophils of the animals of FIG. 1A. Statistical analysis of the data using nonparametric unpaired t-test yielded p values <0.005 for this data.

FIG. 2A is a bar graph demonstrating the E-selectin-independent adhesion of human mammary adenocarcinoma cells SkBr5 to the human endothelioma cell line ECV-304. SkBr5 and HUVEC cells were allowed to adhere for 15 minutes in the continuous presence of MABs and MAB F(ab)₂ fragments (marked in the graph) at 40 pg/ml. Control anti-influenza hemagglutinin MAB H24135 was used at the same concentration. The results represent the percentage (%) of adherent cells in the presence of specific MAB as compared to control anti-influenza hemagglutinin MAB H24135. Results are reported as means±standard error (SE) of five independent experiments.

FIG. 2B is a graph depicting the representative data of the expression of various antigens on the surface of cells in the absence (−) or presence (+) of the inflammatory cytokine, Interleukin-1, as determined by MABs binding in radioimmunoassay (RIA) with monolayer ECV-304 cells. The presence of the following antigens was detected using the MABs indicated: SA-Le^a (MAB NS19-9), CD63 (MAB C029 and ME491), E-selectin (specific MAB anti-ELAM-1, British

Bio-Technology, UK), and anti-influenza virus hemagglutinin negative control (MAB H24135).

FIG. 3 is a graph illustrating the effect of Trp5 substitution with Phe in peptide DLWDWVVGKPAG [SEQ ID NO: 1] resulting in peptide DLWDFVVGKPAG [SEQ ID NO: 63] on binding of SA-Le^a specific MAB NS19-9. Constant amounts of Mab were incubated with increasing amounts of peptides and binding of free antibody to carbohydrate SA-Le^a was measured by enzyme linked immunosorbent assay. Results show competitive inhibition of MAB binding to solid phase SA-Le^a polyacrylamide matrix (SA-Le^a-PAA) by 12-mer peptides DLWDWVVGKPAG (■) [SEQ ID NO: 1] and DLWDFVVGKPAG (▲) [SEQ ID NO: 63] with respect to the MAB binding without peptide (100% of binding) and a negative control unrelated peptide (○).

FIG. 4 is a circular dichroism (CD) spectra comparing dodecapeptides DLWDWWGKPAG (solid line) [SEQ ID NO: 1] and DLWDFVVGKPAG (---) [SEQ ID NO: 63]. The spectra were recorded at 0.51 mg/ml for both peptides.

FIG. 5 is a graph illustrating the inhibition of lung experimental metastases with peptide DLWDFVVGKPAG [SEQ ID NO: 63]. Tumor cells were admixed with the specific or unrelated peptide solution (1 mg per mouse) and animals were inoculated with 1×10⁵ B16F10FtIII tumor cells in 200 µl volume of PBS via tail vein. Results are from 4 experiments (5 mice in each group) are shown. Each dot represents enumerated tumor nodules in one lung in experimental group of C57B1/6 mice treated with the peptide (panel B), control group of C57B1/6 mice treated with unrelated peptide (panel A) and E-selectin knock-out (KO) mice of C57B1/6 background (panel C). Statistical analysis using a nonparametric unpaired t test gave a two-tailed p values <0.008 and 0.009 for animals treated with peptide and E-selectin KO, respectively, as compared to control group. The horizontal bars represent median values and vertical bars denote standard deviation.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the discovery that peptido-mimetics of complex carbohydrate structures block adhesion of tumor cells and leukocytes to endothelial cells. The production of small peptide molecules which mimic complex carbohydrate structures are useful for blocking carbohydrate ligand-cell adhesion molecule interactions involved in metastasis, angiogenesis, and inflammatory responses. Thus, these peptido-mimetics are useful as anti-adhesion therapeutics. The evaluation of complex carbohydrate ligand-adhesion molecule interactions at the molecular level enables the design of peptido-mimetics and possibly other compounds which block these adhesion interactions thereby disrupting the disease processes mediated by them. Further, the ability to generate peptide mimotopes of complex carbohydrate structures enables the use of recombinant peptide library display technology in discovering novel blocking agents for important therapeutic targets which bind carbohydrate ligands. The compositions and methods of this invention are also useful in designing additional therapeutics to inhibit the adhesion interactions required for disease processes.

As used herein, the term "peptido-mimetic" means a peptide or polypeptide that mimics complex carbohydrate conformations and structures. A subset of peptido-mimetics are referred to as "mimotopes". Mimotopes are small peptido-mimetics, generally from 7 to about 15 amino acids in length which mimic complex carbohydrate structures.

including the carbohydrate ligands for endothelial adhesion molecules. Mimotypes are also capable of blocking the ligand-adhesion molecule interaction. Throughout this specification, these terms are used interchangeably. These small peptides also facilitate the study of complex structural/conformational relationships between these ligands and cellular lectins.

A. Peptides of the Invention

A composition of this invention comprises at least one peptido-mimetic of a carbohydrate ligand of an adhesion molecule in a physiologically acceptable carrier. In one embodiment, the adhesion molecule is a selectin, such as E-selectin. In another embodiment, the ligand is a Lewis antigen. Particularly desirable are peptido-mimetics of the Lewis antigens SA-Le^a, SA-LeX, and LeY. The illustrative peptides described below may be modified, as described in more detail herein, to improve anti-adhesion properties and to increase their stability to degradation in vivo.

Desirable compositions of this invention contain one or more of the following peptido-mimetics which mimic the topography of the E-selectin ligand: ASAVNLYIPTQE [SEQ ID NO:84], VYLAPGRISRDI [SEQ ID NO:85], VYLAPGRFSRDI [SEQ ID NO:86], CTSHWGVLSQRR [SEQ ID NO:87], RVLSPESYLGPS [SEQ ID NO:88], RVLSPESYLGPA [SEQ ID NO:89], VGNGVLMGRRG [SEQ ID NO:90], RVLSPESYLGPA [SEQ ID NO:92], GNCRYIGLRQFG [SEQ ID NO:93], DIRVEPGGGYTH [SEQ ID NO:94], APIHTYTGRARG [SEQ ID NO:96], and RHTCVRSCGHDR [SEQ ID NO:97].

Similarly, exemplary peptido-mimetics of SA-Le^a include, without limitation, VGIWSWSEGSR [SEQ ID NO: 102], RCSVGVPFTMES [SEQ ID NO:103], QDGVWEHVLEGG, [SEQ ID NO:104], DLWDWV-VGKPAG [SEQ ID NO:1], VELSGRGGLCTW [SEQ ID NO:105], VIGAASHDEDVD [SEQ ID NO:106], TIEPV-LAEMMG [SEQ ID NO:107], DKETFELGLFDR [SEQ ID NO:108], FSGVRGVYESRT [SEQ ID NO:109], PDDAP-MHSTRVE [SEQ ID NO:110], STGLMVDFLEPG [SEQ ID NO: 91], AKTFGLEHGCEA [SEQ ID NO: 95], GGTVEVWSIKGG [SEQ ID NO: 115], DHFSQAGSS-NHH [SEQ ID NO: 116], DDPVTPVIDFGK [SEQ ID NO: 117], and RDGLIDFVVAGT [SEQ ID NO: 118].

As described in the examples below, families of mimics of carcinoma-associated antigens that represent SA-Le^a, in particular, were identified from a combinatorial peptide library using MAb NS 19-9 specific for this carbohydrate structure. One of the peptides, DLWDWVVGKPAG [SEQ ID NO: 1], was selected that specifically competes for binding of MAb for SA-Le^a. This peptide displays an ability to partially inhibit neutrophil recruitment in an acute inflammation model in vivo. As described below, this peptide was analyzed by systematic amino acid replacements to identify optimal conformationally stabilized SA-Le^a mimics with higher affinity using a solid phase peptide array library. Comparison of signal intensities revealed significant differences in MAb binding as a result of substitutions, in particular at the N-terminus. Substitution analysis allowed for delineation of key residues that were sensitive to replacement. MAb NS19-9 discriminated against multiple amino acid substitutions affecting its recognition. Specific residues within this peptide were identified that may contribute to the mimicry of carbohydrate structure by the peptide.

On the other hand MAb NS19-9 could tolerate replacement of the lead peptide sequence by a variety of amino acids. These substitutions did not abrogate binding, suggesting that they did not affect the structural specificity required

for MAb recognition. Different amino acids in themselves can act as structural mimics within an identified peptide and bind through non-specific interactions. The different consensus sequences among the families of peptides identified with the same MAb from the random peptide library or sequences without an obvious consensus characterized in previous studies support the notion that indeed different amino acid residues provide structural similarity. Alternatively, different consensus sequences mimic different topographies of the carbohydrate epitope recognized by the antibody.

The identification of several cross-reactive peptides displaying higher NS19-9 binding further delineate specific residues that may improve upon peptide mimicry of the carbohydrate structure. Several substitutions within the C-terminus, in particular with amino acids containing carboxyl groups that increased MAb binding, were identified suggesting an important role of polar interactions in binding affinity. The strongest signal however resulted from the single amino acid substitution of Phe for the Trp at position 5, creating the SA-Le^a peptidomimetic DLWDFVVGKPAG [SEQ ID NO: 63].

The present invention demonstrates that peptides mimicking SA-Le^a are able to bind surfaces of proteins specific for these structures and thus they can act as antagonists for the recognition of the cognate carbohydrate antigen or ligand. In vivo oligosaccharide dependent reduction of metastasis formation may be a function of the interruption of these interactions. Antagonists interfere with the metastatic process at the level of cellular adhesion and blood vessel formation since E-selectin and SA-LeX are expressed on actively growing blood vessels. Alternatively, peptide mimics act on signal transduction events mediated by selectins and their ligands and the in vivo consequences of selectin-ligand antagonism to the complex signal transduction processes associated with selectin-dependent cell adhesion.

Certain exemplary peptidomimetics of LeY include the peptides TKRPDLIVDPIP [SEQ ID NO:98], DEVRP-DLISTEE [SEQ ID NO: 99], NLRPKYIXLDAD [SEQ ID NO:100], and TLIAFADLVDVI [SEQ ID NO: 101].

Peptides mimicking carbohydrate antigens retain conformational properties of cognate carbohydrate structures and can block recognition of cells expressing such ligands in vivo. Thereby, they can mediate anti-metastatic functions as demonstrated by blocking of experimental metastasis. Thus, the peptides identified above which mimic the topography of the E-selectin ligand and/or the other Lewis antigens can be employed in pharmaceutical compositions. Such peptido-mimetics are useful in pharmaceutical compositions directed toward the treatment of cancer, and the prevention and/or inhibition of metastases (see, e.g., Example 14). Such peptides may also be used in pharmaceutical treatments to block selectin-dependent interactions, e.g. diminishing the inflammatory response (see, e.g., Example 8).

The above peptido-mimetics and others which may be identified by use of the assays referred to below may be further modified to increase or enhance the stability of such peptides for in vivo use or to enhance the binding abilities of these peptido-mimetics. Peptido-mimetics according to this invention includes peptido-mimetics such as those identified specifically above, which are modified to increase their stability in vivo.

For example, the incorporation of unnatural amino acids (e.g., D configuration amino acids) at the N or C termini, the most frequent peptide degradation sites, may improve the pharmacological properties of the peptides, without loss of

be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to associate with the peptides of this invention.

Specialized computer programs that may also assist in the process of selecting fragments or chemical entities similar to the peptides, or entities which can interact with the peptides and thus mimic the receptor, include the GRID program available from Oxford University, Oxford, UK. [P. J. Goodford, "A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules", *J. Med. Chem.*, 28:49-857 (1985)]; the MCSS program available from Molecular Simulations, Burlington, Mass. [A. Miranker and M. Karplus, "Functionality Maps of Binding Sites: A Multiple Copy Simultaneous Search Method", *Proteins: Structure, Function and Genetics*, 11:29-34 (1991)]; the AUTODOCK program available from Scripps Research Institute, La Jolla, Calif. [D. S. Goodsell and A. J. Olsen, "Automated Docking of Substrates to Proteins by Simulated Annealing", *Proteins: Structure, Function, and Genetics*, 8:195-202 (1990)]; and the DOCK program available from University of California, San Francisco, Calif. [I. D. Kuntz et al, "A Geometric Approach to Macromolecule-Ligand Interactions", *J. Mol. Biol.*, 161:269-288 (1982)], software such as Quanta and Sybyl, followed by energy minimization and molecular dynamics with standard molecular mechanics force fields, such as CHARMM and AMBER. Additional commercially available computer databases for small molecular compounds include Cambridge Structural Database, Fine Chemical Database, and CONCORD database [for a review see Rusinko, A., *Chem. Des. Auto. News*, 8:44-47 (1993)].

Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound. Assembly may proceed by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure of the peptido-mimetic of this invention. Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include the CAVEAT program [P. A. Bartlett et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", in *Molecular Recognition in Chemical and Biological Problems*, Special Pub., Royal Chem. Soc. 78, pp. 182-196 (1989)], which is available from the University of California, Berkeley, Calif.; 3D Database systems such as MACCS-3D database (MDL Information Systems, San Leandro, Calif.) [see, e.g., Y. C. Martin, "3D Database Searching in Drug Design", *J. Med. Chem.*, 35:2145-2154 (1992)]; and the HOOK program, available from Molecular Simulations, Burlington, Mass.

Compounds that mimic a peptide of this invention or a ligand of the peptides may be designed as a whole or "de novo" using either an empty active site or optionally including some portion(s) of a known ligand(s). Suitable methods describing such methods include the LUDI program [H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", *J. Comp. Aid. Molec. Design*, 6:61-78 (1992)], available from Biosym Technologies, San Diego, Calif.; the LEGEND program [Y. Nishibata and A. Itai, *Tetrahedron*, 47:8985 (1991)], available from Molecular Simulations, Burlington, Mass.; and the LeapFrog program, available from Tripos Associates, St. Louis, Mo. Other molecular modelling techniques may also be employed in accordance with this invention. See, e.g., N. C. Cohen et al, "Molecular Modeling Software and Methods for Medicinal Chemistry", *J. Med. Chem.*, 3:883-894

(1990). See also, M. A. Navia and M. A. Murcko, "The Use of Structural Information in Drug Design", *Current Opinions in Structural Biology*, 2:202-210 (1992). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the peptide of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, e.g., P. S. Farmer, *Drug Design*, Ariens, E. J., ed., Vol. 10, pp 119-143 (Academic Press, New York, 1980); U.S. Pat. No. 5,331,573; U.S. Pat. No. 5,500,807, C. Verlinde, *Structure*, 2:577-587 (1994); and I. D. Kuntz, *Science*, 25:1078-1082 (1992). The model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Thus, using these computer evaluation systems, a large number of compounds may be quickly and easily examined and expensive and lengthy biochemical testing avoided. Moreover, the need for actual syntheses of many compounds is effectively eliminated. Once identified by the modelling techniques, the proposed "new antibacterial" compound may be tested for bioactivity using standard techniques, such as the assays of the examples below.

The invention is further described in detail by reference to the following, experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLE 1

Screening of a Random Peptide Library

To develop novel molecules to inhibit the adhesion of human adenocarcinoma cells to EC and, ultimately, to inhibit metastasis in vivo, peptides were derived from a 12-mer random peptide library. A diverse library of random dodecapeptides, displayed as flagellin-thioredoxin fusion proteins (FLITRX) on the surfaces of *E. Coli* bacterial cells, was obtained from Invitrogen (Carlsbad, Calif.) [LaVallie et al., 1993, *Bio/Technology*, 11:187-193]. This library enables efficient isolation of bacteria displaying peptides with affinity to immobilized antibodies or to other binding proteins. The use of this library offered advantages over phage display in which the level of expression of phage coat protein genes is low and the selected peptides are usually unconstrained molecules with many degrees of conformational freedom. Moreover, no phage infection or isolation steps are necessary using the FLITRX fusion protein system. Such highly diverse peptide libraries offer many distinct advantages over difficult chemical or enzymatic synthesis of complex carbohydrates, providing for the inexpensive and rapid identification and optimization of novel ligands.

Specifically, an aliquot of the FLITRX library containing at least 2×10^{10} cells to ensure full representation of peptides, was grown to saturation for 15 hours in IMC/amp 100 medium (M9 medium containing 1 mM $MgCl_2$ supplemented with 0.5% glucose, 0.2% casamino acids and 100 $\mu g/ml$ ampicillin). The expression of thioredoxin with incorporated 12-mer peptide sequence was induced by further 6 hours of incubation of a culture of 10^{10} cells, diluted 1:25 with fresh IMC/amp 100 medium containing 100 $\mu g/ml$ tryptophan. The induced bacteria were panned on a MAb-coated tissue culture (20 $\mu g/ml$) plate followed by blocking with 1% nonfat milk containing 150 mM NaCl and 1%

DESIGN (LUDI) program (Biosym Technologies, San Diego, Calif.) [Bohm 1992, *J. Comput. Aided Mol. Des.* 6:593-606]. This program searches a molecular library for fragments representative of the amino acids in the target peptide sequence. The program then positions the fragments within the combining site devoid of steric conflicts.

Previously, a molecular basis for interaction of LeY tetrasaccharide and MAb BR55-2 binding site was elucidated using molecular modeling [Blaszczuk-Thurin et al., 1996, *Protein Engineering* 9:101-113]. Modeling studies of the APWLYAGP [SEQ ID NO: 83] sequence mimicking LeY carbohydrate in the combining site of the anti-LeY antibody B3 [Murali and Kieber-Emmons, 1997, *J. Molec. Rec.*, 10:269-276] indicate that the putative contact residues APWLYA of SEQ ID NO: 83 adopt a turn conformation. Such a conformation is also projected for residues displayed in the constrained library.

In order to establish how the putative APWLY sequence of SEQ ID NO: 83 mimics LeY binding to B3, the pentapeptide sequence was "fitted" into the B3 combining site using the LUDI program. Using this program, the APWLY sequence of SEQ ID NO: 83 was modeled such that the Trp (W), Tyr (Y), Leu (L) and Ala (A) residues occupied relative positions as the identified LUDI fragments. Judicious positioning relied upon intermolecular interaction calculations in which several potential binding modes of the peptide were ranked according to the stability of the complex.

In the most stable conformation, the AP residues occupied a similar position to the LeY GlcNAc residue. This positioning indicates that the proline residue mimics the spatial position of the glucose unit of GlcNAc, while the Ala methyl group is positioned similarly to the terminal methyl group of GlcNAc's N-acetyl. The Trp residue occupies a volume associated with the Fuca 1,3 moiety, and the Leu residue occupies the volume of the β Gal moiety and has the hydrophobic interaction of β Gal. The Tyr residue occupies a position not associated with LeY binding to B3. The computer modeling disclosed that the low energy binding mode conformation adopts a turn region similar to that observed for the YPY motif in binding to ConA [Kaur et al., 1997, *J. Biol. Chem.* 272:5539-5543]. This conformation lends itself to the Tyr residue of the peptide to potentially interact with several residues in CDR2 of the heavy chain of B3 that include Asp H53, Ser H52, Ser H55, or Ser H56. These residues are different in BR55-2, which does not bind the monovalent APWLYGPA [SEQ ID NO: 121] peptide in a series of ELISA assays.

More importantly, energy optimization of the positioned peptide identified similar functional groups within the B3 combining site in contact with the peptide and carbohydrate tetrasaccharide core of LeY. Therefore, this analysis provides a strategy for determining the molecular basis for antigenic mimicry of particular motifs, providing a unique perspective of how a peptide sequence fits into the antibody or a receptor combining site, competing with a native antigen. This approach also enables the design of therapeutic compounds which more effectively compete with the native carbohydrate ligand for binding to cell adhesion molecules.

The above-disclosed approach was further extended to determine the types of motifs that bind to BR55-2 and whether such motifs could be isolated with BR55-2 from a peptide phage screen. Identification of such peptides as motifs would be a first step in to improving upon antigenic mimicry for LeY. Search of the LUDI database using the modeled structure of BR55-2 identified the motifs YPY, YRY and WRY, which are known to mimic various carbo-

hydrate subunits, as interacting with BR55-2 and also identified a non planar-X-planar type motif, FSLLW [SEQ ID NO: 114], as possibly interacting. Thus, non-overlapping residue types were identified using the LUDI program as in the B3 studies. A computer-generated space filling model was generated which depicted the identification and placement of an optimized "FSLLW" amino acid motif in the combining site of anti-LeY monoclonal antibody BR55-2 in contrast to LeY positioned in the BR55-2 combining site. The topological similarity is very good. This peptide competes with LeY for BR55-2 binding. These data further demonstrate another method to develop peptide mimotopes that are specific. This method may be extended to identify motifs mimicking SA-LeX and SA-Le^Y using the crystal structure of the lectin domain of E-selectin in optimizing respective mimotopes.

EXAMPLE 6

Identification of Sequences Critical for MAb Binding and an SA-Le^Y Mimic with Higher Antibody Binding Affinity

To analyze amino acid residues that are critical for NS19-9 recognition, an array library of 163 unique peptides was generated by systematic amino acid replacement in which each position of the starting peptide DLWDWV-VGKPAG [SEQ ID NO: 1] was replaced by other L-amino acids. In addition, peptides were synthesized with simultaneous incorporation of multiple amino acids or with truncation of specific regions.

The peptide array of 163 unique peptides was generated by substituting all amino acids for each individual amino acid in lead peptide (DLWDWVVGKPAG [SEQ ID NO: 1] identified by combinatorial library panning with MAb NS19-9. An array of synthetic 12-mer peptides was synthesized using 90x130 mm polyethylene glycol-modified cellulose membrane functionalized with approximately 4 nmole/mm² amino groups, manufactured by Abimed (Lagenfeld, Germany). Standard Fmoc chemistry was used according to the manufacturer's instructions [Frank, R., 1992, *Tetrahedron* 48, 9217-9332]. The protected and activated amino acids were spotted using an Abimed ASP 422 robotic arm. All washing, dyeing and deprotection steps were done manually. The activated C-terminal amino acids were spotted leaving 10 mm space in each direction, at the concentration of 0.5 M in N-methyl pyrrolidone. A volume of 0.5 ml provides spot of 7-8 mm in diameter. Activation of the amino acids with dicyclohexyl-carbodiimide and N-hydroxy-benzotriazole was done 30 minutes before spotting. After each coupling cycle, the paper was washed with 12% acetic anhydride dissolved in N,N'-dimethylformamide (DMF) twice for a total of 10 min to endcap all unreacted amino groups. Repetitive removal of the Fmoc groups was achieved by two treatments with 20% piperidine in DMF for 5 and 10 min, respectively. The second and consecutive amino acids were coupled in a 1.1 molar excess, and were spotted 3-4 times depending upon the outcome of the bromophenol blue assay of the couplings.

After the coupling and deprotection steps, the membrane was washed thoroughly with DMF and ethanol, dried and stained with bromophenol blue dissolved in DMF. After successful coupling the paper remains colorless; after successful deprotection steps the peptide dots turn deep blue. The coupling steps were repeated until all peptide spots remained colorless. The N-terminal amino acids at the end of the syntheses remained uncapped. Final removal of the side-chain protecting groups was performed by washing the

Array analysis failed to reveal significant differences in the binding intensities between the peptides substituted at different positions, suggesting that single substitution at any position in this region with carboxyl groups can enhance the interaction with the MAb binding site. In addition, substitutions with Ile, Ala and Ser also improved MAb binding. Similarly, the simultaneous replacement of several residues with clusters of amino acids upstream from position 6 demonstrated enhanced binding. The highest intensity signal was, however, observed with peptide DLWDEVVGKPAG [SEQ ID NO:63] containing a single substitution at position 5 with Phe.

Table 5 lists the amino acid substitutions within peptide DLWDWVVGKPAG [SEQ ID NO: 1] that increase the binding of MAb NS19-9 using the peptide array.

TABLE 5

Peptide Sequence	SEQ ID NO
DLWDFVVGKPAG	63
DLWDWVIGKPAG	64
DLWDWVVKAPAG	65
DLWDWVVSKPAG	66
DLWDWVVEKPAG	67
DLWDWVVDPKAG	68
DLWDWVVGEKAG	69
DLWDWVVGDKAG	70
DLWDWVVGKEAG	71
DLWDWVVGKDAG	72
DLWDWVVGKPDG	73
DLWDWVVGKPAD	74
DLWDWVKEKPAG	75
DLWDWVLAKPAG	76
DLWDWVVGEDAG	77
DLWDWVVVGKPEK	78
DLWDWVVKEEPAG	79
DLWDWVVVGKDEK	80
DLWDWVVGEDEK	81
DLWDWVKEEDEK	82

A distinct pattern of substitutions that led to increased or abolished signal intensities with respect to the C- and N-terminus suggests that the region close to the N-terminus might contribute to the specificity of the interaction with NS19-9. Amino acids close to the C-terminus appear to add significantly to the affinity of ligand binding.

EXAMPLE 7

Inhibition of Binding of MAb to the Carbohydrate with Synthetic Peptides Mimicking SA-Le^a Structure

DLWVDWVVGKPAG [SEQ ID NO:1] and DLWDFVVGKPAG [SEQ ID NO:63] were chemically synthesized and tested for their ability to compete to immobilized synthetic SA-Le^a-PAA neoglycoprotein. To determine whether peptide SEQ ID NO: 1 and peptide SEQ ID NO: 63 are true mimics of SA-Le^a, dose-response experiments were carried out in order to determine the concentration of peptides required for blocking of 50% of MAb binding to the native carbohydrate antigen (IC₅₀), as determined by competition ELISA (Example 3). Both peptides SEQ ID NOS: 1 and 63 blocked the binding of MAb NS 19-9 to the constant amount of carbohydrate antigen in a dose-dependent manner. The IC₅₀ for peptide SEQ ID NO: 1 blocking of MAb-SA-Le^a binding was 700 μM. Peptide SEQ ID NO: 63 exhibited a more pronounced dose-dependent inhibition of the MAb-SA-Le^a binding as compared with peptide SEQ ID NO: 1 as demonstrated by the calculated IC₅₀ value of 70 μM for peptide SEQ ID NO: 63.

Without wishing to be bound by theory, these data suggest that the peptide sterically interferes with MAb binding to carbohydrate antigen. The sequences DLWDWVVGKPAG [SEQ ID NO: 1] and DLWDFVVGKPAG [SEQ ID NO:63] represent solvent-accessible epitopes and the peptides represent cognate determinants for the antibody. No measurable blocking of anti-Le^a MAb NS 19-9 binding was found with non-related peptide, indicating that the inhibitory effects of the native sequences are due to specific effects.

EXAMPLE 8

Inhibition of Neutrophil Recruitment in an Acute Inflammation Model In Vivo by a Peptido-Mimetic of SA-Le^a

The accumulation of neutrophils is a characteristic feature of acute and chronic inflammatory disease, and early steps in the recruitment of these cells to the site of inflammation depends upon E-selectin-mediated interaction. Thus, inhibition of neutrophil recruitment in vivo is an important test of the ability of potential therapeutic agents to inhibit E-selectin-mediated events. SA-Le^a interaction with E-selectin is not relevant for adhesion of neutrophils, since neutrophils do not express SA-Le^a on their surfaces. However, tumor cells have been demonstrated to prefer SA-Le^a over SA-Le^x in mediating E-selectin-dependent adhesion interactions with endothelial cells. Because SA-Le^a binds to E-selectin, the adhesion-blocking ability of an SA-Le^a peptido-mimetic was measured in a neutrophil-recruitment in vivo model of acute inflammation. Thus, this assay demonstrates that the administration of a SA-Le^a mimicking molecule inhibits neutrophil recruitment, i.e., diminishes the influx of neutrophils into chemically irritated peritoneum in vivo.

The bioactivity of the 12-mer peptide SEQ ID NO: 1 which mimics the SA-Le^a carbohydrate structure was determined by administering Zymosan™ intraperitoneally (i.p.) into mice [see, e.g., Martens et al. 1995, *J. Biol. Chem.* 270:21129-21136; Rao et al. 1994, *J. Biol. Chem.* 269:19663-19666], followed three hours later by an intravenous (i.v.) injection of peptide SEQ ID NO: 1 (1 mg). Neutrophils were harvested and counted one hour later. As shown in FIG. 1A, peptide treatment significantly (P<0.001) reduced the number of neutrophils in peritoneal lavage fluids. Control experiments using the same dose of "scrambled" peptide sequence, which did not bind to NS19-9 MAb, exhibited no decrease in neutrophil influx relative to PBS-injected mice.

To confirm these results, myeloperoxidase (MPO), activity, which is an enzymatic marker for neutrophils, was measured spectrophotometrically as an absorbance rate in the homogenates of collected cells [Bradley et al., 1982, *J. Invest. Derm.* 78:206-209]. Significant reduction of enzymatic activity (P<0.005) was observed in parallel with decreased neutrophil numbers assessed by total neutrophil count (FIG. 1B). The reduction in enzyme activity is apparently due to reduction in neutrophil recruitment in mice treated with peptide mimicking E-selectin ligand SA-Le^a. This peptide inhibits E-selectin function in vivo. Although peptide SEQ ID NO: 1 exhibited relatively low-affinity binding to MAb in vitro, IC₅₀ data from in vitro competition assays (Example 3) may not be relevant to the in vivo situation since the E-selectin blocking effect was clearly observed despite the low affinity demonstrated in vitro.

EXAMPLE 9

Peptido-Mimetics of E-Selectin Ligand

To identify peptides mimicking E-selectin ligand that may involve other than carbohydrate epitopes of the natural

in multiple in vitro studies. An in vivo experimental metastatic model which permits investigation of SA-Le^a supported adhesion of tumor cells to lung endothelium is performed as follows:

B16F10 murine melanoma cells do not naturally express E-selectin ligands SA-LeX or SA-Le^a as demonstrated by FACS analysis, and are syngeneic with C57B1/6 haplotype (American Type Tissue Collection, Rockville, Md.). To manipulate these cells to express the SA-Le^a structure on the tumor cell surface, the B16F10 cells were stably transfected with pCDNA-FTIII using Effectene (Qiagen, Chatsworth, Calif.) as recommended by the manufacturer. Plasmid pCDNA-FTIII is prepared by cloning HindIII and NotI-digested α 1-3/4-fucosyltransferase cDNA (FTIII) obtained from the π H3M vector containing FTIII cDNA (Brian Seed, Massachusetts General Hospital, Boston, Mass.) was cloned into pCDNA3(neo) vector. FTIII fucosyltransferase is specific for both type 1 and 2 lactoseries oligosaccharide acceptor substrates and thus is capable of synthesizing both SA-Le^a and SA-LeX, respectively. The resulting cell line, B16F10FTIII, expresses SA-Le^a carbohydrate structure as demonstrated by flow cytometry analysis using MAb NS19-9 as compared to the parental B16F10, which did not show staining with this antibody.

The transfected cells were grown in the presence of G418 (500 μ g/ml) (Gibco-BRL, Grand Island, N.Y.) for 10 days. To ensure the homogeneity of the transfected cells with respect to the expression of SA-Le^a, the cells were subjected to cell sorting using SA-Le^a specific MAb NS19-9 followed by FITC-conjugated goat anti-mouse immunoglobulin. The resulting cell line B16F10FTIII appeared to express SA-Le^a but not SA-LeX as assessed by FACS (not shown), suggesting that type 1 but not type 2 acceptors were available within the cells. Thus, the generated cell line made a suitable model to determine the role of SA-Le^a in the metastatic process since the tumor cells are devoid of SA-LeX. The tumorigenic dose for the C57B1/6 syngeneic tumor cells was established by i.v. injection of various numbers of cells. A 1×10^5 dose was chosen for further experiments as countable lung metastases were observed after i.v. injection of 1×10^5 of B16F10FTIII cells expressing SA-Le^a after 21 days.

The role of tumor cell adhesion to vascular EC via E-selectin and its ligand SA-Le^a interaction in metastasis formation was established in vivo in two ways. First, to directly assess the role of E-selectin in tumor colonization in vivo, the ability of B16F10 murine melanoma cells expressing SA-Le^a to colonize in the lung of E-selectin KO mice of C57B1/6 background [Staite, N. D. et al, 1998, *Blood*, 88: 2973-2979, and kindly provided by Dr. Daniel Bullard (University of Alabama at Birmingham, Ala.)] was determined in parallel with wild-type six- to 8-week-old C57B1/6 female mice (Jackson Laboratory, Bar Harbor, Me.). KO mice lack E-selectin expression.

B16F10FTIII cells positive for SA-Le^a were grown in vitro in Iscove's culture medium supplemented with 10% FBS for 1 week before injecting into mice. Cells were collected and washed twice in Iscove's medium without serum and suspended in PBS. One $\times 10^5$ tumor cells in a volume of 200 μ l in PBS were inoculated by intravenous (i.v.) route via tail vein. To test the effect of the peptide, animals were inoculated with a single dose of peptide at the time of tumor challenge. One mg of peptide was admixed with the tumor cells and together injected via i.v. route. Control animals received injection of tumor cells admixed with the same amount of unrelated peptide. Mice were euthanized after 3 weeks following tumor cells injection and lung and other organs were examined under dissecting microscope for the presence of tumor nodules. The lungs were excised and the number of nodules was enumerated for each animal without fixation of the lungs. Data were evalu-

ated for statistical significance using a nonparametric unpaired two-tailed t test. The results are shown in FIG. 5.

Mice of both strains received i.v. injection of 1×10^5 B16F10FTIII tumor cells and mice were examined 3 weeks later. Only 20% of E-selectin deficient animals injected with tumor cells developed small numbers of lung metastasis while the rest of the E-selectin KO mice showed no detectable lung tumor nodules. Statistical analysis gave a P values < 0.009 for E-selectin KO as compared to the control group (FIG. 4, A and C), respectively. Small nodules were observed in a few E-selectin KO mice that developed tumors whereas all animals in the control group developed multiple metastasis and some of them died earlier than 3 weeks. The results demonstrate that lung metastasis of tumor expressing SA-Le^a antigen is completely abrogated in most of the genetically manipulated mice that lack expression of E-selectin, highlighting the critical role of E-selectin in mediating carcinoma metastasis in vivo.

To further test the hypothesis that SA-Le^a expression supports adhesion of tumor cells to E-selectin on EC, the inhibitory effect of peptide mimicking SA-Le^a antigenic structure DLWDFVVGKPAG [SEQ ID NO: 63] on lung colonization of B16F10FTIII cells was tested. One $\times 10^5$ tumor cells expressing SA-Le^a were admixed with a solution containing 1 mg of the peptide DLWDFVVGKPAG [SEQ ID NO: 63], followed by administration of the mixture to groups of mice. Because peptides in general show rather short half-life in mouse serum, in the peptide inhibition studies in vivo the peptide was admixed with the tumor cells to sustain the highest transient concentration of peptide at the time of tumor cell arrival into the lung capillary system. Animals were euthanized after 21 days following tumor challenge and the number of metastasis was enumerated in each lung whereas, no metastatic growth was detected in the liver.

Administration of the peptide DLWDFVVGKPAG [SEQ ID NO: 63] abrogated on average 50% lung colonization of the B16F10FTIII induced tumor nodules developed in control animals; some mice being completely devoid of tumor nodules (FIG. 4B). The injection of the peptide 1 hour prior to tumor cells did not influence the rate of metastases formation in comparison with the peptide administered together with tumor cells. Animals treated with peptide showed metastases ranging from 0 to 20 per lung (median 9.9), whereas, animals in the control group developed multiple tumor nodules with the number of metastases per mouse ranging from 3 to 40 per lung (median 20.7) (FIG. 4B and 4A), respectively). The difference was highly statistically significant ($p < 0.008$). In addition, B16F10FTIII cells in C57B1/6 mice developed large tumor masses with diffused infiltration of tumor cells and some mice died before the termination of the experiment (median 16 days). Mice that developed metastases, despite treatment with SA-Le^a mimicking peptide, showed the presence of small tumor nodules and all survived the 3 week observation time.

These results suggest that the synthetic structural conformer mimicking SA-Le^a antigen is able to significantly block the adhesion of tumor cells to vascular endothelium at the early stages of the multistep process, thus reducing tumor metastases. This finding strongly suggests that the interaction of SA-Le^a carbohydrate tumor-associated antigen with E-selectin expressed on vascular EC is an important step in establishing tumor metastasis.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims to be construed to include all such embodiments and equivalent variations.

ERROR (15)

US 6,960,566 B1

69

70

-continued

<210> SEQ ID NO 121
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: peptido-mimetic of a Lewis antigen

<400> SEQUENCE: 121

Ala Pro Trp Leu Tyr Gly Pro Ala
1 5

What is claimed is:

1. A peptide or polypeptide selected from the group consisting of: ASAVNLYIPTQE SEQ ID NO:84, VYLAP-GRISRDY SEQ ID NO:85, VYLAPGRFSRDY SEQ ID NO:86, CTSHWGVLSQRR SEQ ID NO:87, RVLSP-ESYLGPS SEQ ID NO:88, VGNGVLMGRRG SEQ ID NO:90, RVLSPESYLGPA SEQ ID NO:92, GNCRYIGL-ROPG SEQ ID NO:93, DIRVEPGGGYTH SEQ ID NO:94, APIHTYTGRARG SEQ ID NO:96, and RHTCVR-SCGHDR SEQ ID NO:97 which binds to a selectin and blocks binding between said selectin and a Lewis SA-Le^a or SA-LeX antigen.

2. The peptide or polypeptide according to claim 1, which comprises a modification selected from the group consisting of (i) use of one or more D amino acids, (ii) insertion of a moiety which can provide a net positive charge at the N-terminus of said peptide or polypeptide, (iii) insertion of a spacer of greater than 3 amino acids interposed between the N- and C-termini to cyclize the peptide, (iv) insertion of a free hydroxyl on the C-terminus, (v) insertion of an amide or imide on the C-terminus, and (vi) insertion of a sequence of one or up to about 15 additional amino acids on the C-terminus.

3. A peptide or polypeptide selected from the group consisting of VGIWSVVSEGSR SEQ ID NO:102, RCS-

VGVPFTMES SEQ ID NO: 103, QDGVWEHVLEGG, SEQ ID NO: 104, DLWDWVVGKPAG SEQ ID NO: 1, VELSGRGGLCTW SEQ ID NO:105, VIGAASHDEDVD SEQ ID NO:106, TIEPVLAEMFMG SEQ ID NO:107, DKETFELGLFDR SEQ ID NO:108, FSGVRGVYESRT SEQ ID NO:109, PDDAPMHSTRVE SEQ ID NO:110, STGLMVDFLEPG SEQ ID NO:91, AKTFGLEHGCEA SEQ ID NO:95, GGTVEVWSIKGG SEQ ID NO:115, DHFSQAGSSNHH SEQ ID NO:116, DDPVTPVIDFGK SEQ ID NO:117, and RDGLIDFVVAGT SEQ ID NO:118 which binds to a selectin and blocks binding between said selectin and a Lewis SA-Le^a or SA-LeX antigen.

4. The peptide or polypeptide according to claim 3, which comprises a modification selected from the group consisting of (i) use of one or more D amino acids, (ii) insertion of a moiety which can provide a net positive charge at the N-terminus of said peptide or polypeptide, (iii) insertion of a spacer of greater than 3 amino acids interposed between the N- and C-terminus to cyclize the peptide, (iv) insertion of a free hydroxyl on the C-terminus, (v) insertion of an amide or imide on the C-terminus, and (vi) insertion of a sequence of one or up to about 15 additional amino acids on the C-terminus.

* * * * *

APR 24 2006

ERROR (1)

Exhibit AA



00270

PATENT TRADEMARK OFFICE

WST93AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of) Group Art Unit: 1632
Magdalena Blaszczyk-Thurin et al) Examiner:
Appln. No. 09/831,047)
Filed: May 3, 2001)
For: COMPOSITIONS AND METHODS FOR) May 9, 2002
TREATMENT OF CANCER)

Assistant Commissioner for Patents
Office of Initial Patent Examination
Customer Service Center
Washington, DC 20231

REQUEST FOR CORRECTION OF FILING RECEIPT

Sir:

Enclosed is a copy of the Filing Receipt received in the above-identified patent application. An error in the list of Applicant(s) for this application has been noted and correction to same is marked in red on the attached copy of the Filing Receipt (Exhibit A). Copies of the executed Declaration and Power of Attorney forms (Exhibit B) are attached to show the correct names of the Applicants. Further, Applicants and the undersigned attorney note that the Filing Receipt indicates that there are a total of (41) independent claims in this application. Copies of Applicants Fee Transmittal Letters (Exhibit C) are attached showing the total number of independent claims paid for in this application.

CERTIFICATE UNDER 37 CFR §1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: the Assistant Commissioner for Patents, Washington, DC 20231 on May 9, 2002.

Signature

Typed or printed name

Debra N. Gerstemeier

APR 20 2006

Applicants respectfully request that the official filing receipt for this application be corrected to reflect the correct names of the Applicants as well as the correct number of independent claims.

The Director of the U. S. Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for the Applicants

By Mary E. Bak
Mary E. Bak
Registration No. 31,215
Spring House Corporate Center
Box 457
Spring House, PA 19477
Telephone: (215) 540-9206
Telefacsimile: (215) 540-5818



00270

PATENT TRADEMARK OFFICE PTO/SB/21 (08-00)
Approved for use through 10/31/2002. OMB 0651-0031

U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	09/831,047	
	Filing Date	05/05/2001	
	First Named Inventor	Magdalena Blaszczyk-Thurin et al	
	Group Art Unit	1632	
	Examiner Name		
Total Number of Pages in This Submission	13	Attorney Docket Number	WST93AUSA

ENCLOSURES (check all that apply)		
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment / Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Response to Missing Parts/ Incomplete Application <input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Assignment Papers (for an Application) <input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____	<input type="checkbox"/> After Allowance Communication to Group <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to Group (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) (please identify below): 2 pgs. Request for Correction of Filing Receipt w/Exhibits A, B, and C
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT	
Firm or Individual name	Mary E. Bak, Esquire Howson and Howson
Signature	<i>Mary E. Bak</i>
Date	<i>May 9, 2002</i>

CERTIFICATE OF MAILING			
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on this date: 05/09/2002			
Typed or printed name	Debra N. Gerstemeier		
Signature	<i>Debra N. Gerstemeier</i>	Date	<i>5-9-2002</i>

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

APR 20 2006

PATENT
 Serial No. 09/831047 Doc. No. WST93A01A Atty/Sec MBS/ly Date 5-9-2002
 Inventor Marydona Bloszczyk-Thurman Client WST
 Title Compositional Methods for Treatment of Cancer
 The following has been received in the U.S. Patent and Trademark Office on the date stamped hereon:
 — pp. Spec., — Claims, — Abstract — pp. Amendment: OA dtd —
 — pp. Declaration/Power of Attorney — pp. Response: OA dtd —
 — pp. Verified Statement (Small Entry) — pp. Transmittal Letter —
 — shts. Informal Drawings — ☒ 1 Issue Fee —
 — shts. Formal Drawings — Notice of Appeal & Fee —
 — pp. Assignment — Check # — for \$ —
 — pp. Preliminary Amendment — ☒ 2 (2 figures) for correction
 — pp. Extension of Time — ☒ 1 (1 fig) Request for Exhibits
 — pp. Information Disclosure Statement with PTO-1449 and — references — A, B, C.
 The Patent and Trademark Office is respectfully requested to place its stamp on this postal card and place it in the outgoing mail.

Respectfully,
 HOWSON AND HOWSON
 MBS

APR 20 2006

PATENT

Serial No. 09/831047 Doc. No. 2573A01A Atty/Sec MBS/ly Date 5-9-2002

Inventor Margaret Blaszczak-Thurman Client W.S.

Title Compositions and Methods for Treatment of Cancer

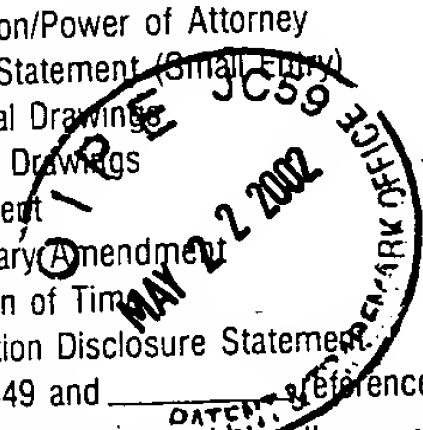
The following has been received in the U.S. Patent and Trademark Office on the date stamped hereon:

☐ pp. Spec., ☐ Claims, ☐ Abstract ☐ pp. Amendment: OA dtd ☐
☐ pp. Declaration/Power of Attorney ☐ pp. Response: OA dtd ☐
☐ pp. Verified Statement (Small Entity) ☒ 1 pp. Transmittal Letter
☐ shts. Informal Drawings ☐ Issue Fee
☐ shts. Formal Drawings ☐ Notice of Appeal & Fee
☐ pp. Assignment ☐ Check # ☐ for \$ ☐
☐ pp. Preliminary Amendment ☒ 2 pp. Request for Correction
☐ pp. Extension of Time ☒ of Filing Request of Exhibits
☐ pp. Information Disclosure Statement ☐ A, B & C.
 with PTO-1449 and ☐ References

The Patent and Trademark Office is respectfully requested to place its stamp on this postal card and place it in the outgoing mail.

Respectfully,
HOWSON AND HOWSON

MBS





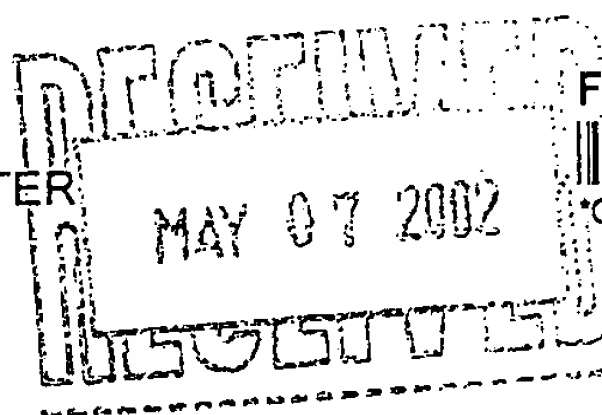
UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

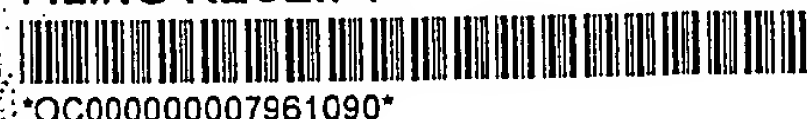
APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/831,047	05/03/2001	1646	814	WST93AUSA	5	41	010

CONFIRMATION NO. 8220

00270
HOWSON AND HOWSON
ONE SPRING HOUSE CORPORATION CENTER
BOX 457
321 NORRISTOWN ROAD
SPRING HOUSE, PA 19477



FILING RECEIPT



OC000000007961090

Date Mailed: 04/30/2002

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Filing Receipt Corrections, facsimile number 703-746-9195. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).

Applicant(s)

Magdalena Blaszczyk-Thurin, Philadelphia, PA;

Thomas Kieber-Emmons, Newtown Square, PA

Domestic Priority data as claimed by applicant

THIS APPLICATION IS A 371 OF PCT/US99/26277 11/05/1999
WHICH CLAIMS BENEFIT OF 60/107,478 11/06/1998

Foreign Applications

Projected Publication Date: Not Applicable, filed prior to November 29, 2000

Non-Publication Request: No

Early Publication Request: No

** SMALL ENTITY **

Title

Compositions and methods for treatment of cancer

Preliminary Class

Exhibit A
APR 20 2006

Attorney's Docket No.: WST93AUSA

TRANSMITTAL LETTER TO THE U.S. ELECTED OFFICE
(EO/US) - ENTRY INTO NATIONAL STAGE UNDER 35 USC 371PCT/US99/26277

International Application No.

5 November 1999

International Filing Date

6 November 1998

Priority Date Claimed

COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

Title of Invention

Magdalena Blaszczyk-Thurin and Thomas Kieber-Emmons

Applicant(s) for EO/US

Box PCT
Assistant Commissioner for Patents
Washington, DC 20231
Attn: EO/US

Sir:

Applicant herewith submits to the United States Elected Office
(EO/US) the following items under 35 USC 371:

- (1) This express request to immediately begin national examination procedures (35 USC 371(f)).
- (2) A copy of the cover sheet for the published International Application along with a copy of the specification as filed: 106 pages, including 7 pages of claims, 5 sheets of drawings, 36 pages of Sequence Listing, and a copy of the 1 page International Search Report.
- (3) a copy of the 5 page Request form.
- (4) a first Preliminary Amendment for entry prior to calculation of the filing fees.
- (5) our check in the amount of \$796.00, covering the basic national fee as set forth in 37 CFR 1.492(a)(1) and based on the first Preliminary Amendment (39 total claims; 10 independent; and no multiple dependent).

Express Mail No. ET 033 649 102 US

- (6) A Second Preliminary Amendment.
- (7) Our check in the amount of \$18.00, covering the extra claim fees after entry of the second Preliminary Amendment (41 total claims; 10 independent; and no multiple dependent).
- (8) Two (2) (4 pages) executed Combined Declaration and Power of Attorney forms.
- (9) A 36 pages Sequence Listing (provided in specification).
- (10) A 3.5" computer-readable diskette.
- (11) A 1 page Statement under 37 CFR §1.821(f) and §1.825(a) and (b).

Copies of the following miscellaneous items are also enclosed:

- (12) Copy of the 4 page Demand for International Preliminary Examination.
- (13) Copy of the 4 page Written Opinion.
- (14) Copy of the 5 page International Preliminary Examination Report.

Please charge any additional fees which may be required to effect entry into the National Phase and credit any overpayment to Deposit Account No. 08-3040.

Please direct all communications concerning this application to the undersigned.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for the Applicants

By Mary E. Bak
Mary E. Bak
Registration No. 31,215
Spring House Corporate Center
Box 457
Spring House, PA 19477
Telephone: (215) 540-9206
Telefacsimile: (215) 540-5818

DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER, the specification of which is attached hereto and was filed as PCT International Patent Application No. PCT/US99/26277, on November 5, 1999.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed	Certified Copy Attached?	
(Number)	(Country)	(MM/DD/YYYY)	—	Yes	No

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

60/107,478
 (Application Number)

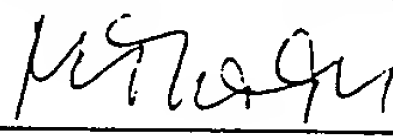
November 6, 1998
 (Filing Date, MM/DD/YYYY)

I hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: STANLEY B. KITA, Registration No. 24,561; GEORGE A. SMITH, JR., Registration No. 24,442; WILSON OBERDORFER, Registration No. 17,379; MARY E. BAK, Registration No. 31,215, CATHY A. KODROFF, Registration Number 33,980, HENRY HANSEN, Registration No. 19,612, and WILLIAM BAK, Registration Number 37,277.

Address all telephone calls to Mary E. Bak at telephone no. (215) 540-9206. Address all correspondence to HOWSON AND HOWSON, Spring House Corporate Center, P. O. Box 457, Spring House, Pennsylvania 19477.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: Magdalena Blaszczyk-Thurin

Inventor's signature  3/29/01
Date

Residence: Philadelphia, Pennsylvania 19104

Citizenship: United States of America

Post Office Address: 28 University Mews, Philadelphia, Pennsylvania 19104

Full name of second inventor: Thomas Kieber-Emmons

Inventor's signature _____ Date _____

Residence: Newtown Square, Pennsylvania 19073

Citizenship: United States of America

Post Office Address: 3231 Saw Mill Road, Newtown Square, Pennsylvania 19073

TRANSMISSION VERIFICATION REPORT

TIME : 01/06/2005 10:54
NAME : HOWSON AND HOWSON
FAX : 2155405818
TEL : 2155409200
SER. # : BROD4J475241

DATE, TIME 01/06 10:51
FAX NO./NAME 17037469195
DURATION 00:03:19
PAGE(S) 14
RESULT OK
MODE STANDARD
ECM

PTO/SB/21 (09-04)

Approved for use through 07/31/2006. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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**TRANSMITTAL
FORM**

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission

14

Application Number	09/831,047
Filing Date	May 3, 2001
First Named Inventor	Błaszczuk-Thurin et al.
Art Unit	1653
Examiner Name	S. Snedden
Attorney Docket Number	WST93AUSA

ENCLOSURES (Check all that apply)

- ☐ Fee Transmittal Form
☐ Fee Attached
☐ Amendment/Reply
☐ After Final
☐ Affidavits/declaration(s)
☐ Extension of Time Request
☐ Express Abandonment Request
☐ Information Disclosure Statement

☐ Certified Copy of Priority Document(s)
☐ Reply to Missing Parts/ Incomplete Application
☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53

- ☐ Drawing(s)
☐ Licensing-related Papers

☐ Petition
☐ Petition to Convert to a Provisional Application
☐ Power of Attorney, Revocation
Change of Correspondence Address
☐ Terminal Disclaimer
☐ Request for Refund
☐ CD, Number of CD(s) _____
☐ Landscape Table on CD

- ☐ After Allowance Communication to TC
☐ Appeal Communication to Board of Appeals and Interferences
☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
☐ Proprietary Information
☐ Status Letter
☒ Other Enclosure(s) (please identify below):
3 pp. Second Request for Correction of Filing Receipt with 5 Exhibits

Remarks

Customer No. 00270

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name

HOWSON AND HOWSON

Signature

APR 20 2006

Auto-Reply Facsimile Transmission



TO: Fax Sender at 2155405818

Fax Information
Date Received: 1/6/2005 10:52:37 AM [Eastern Standard Time]
Total Pages: 14 (including cover page)

ADVISORY: This is an automatically generated return receipt confirmation of the facsimile transmission received by the Office. Please check to make sure that the number of pages listed as received in Total Pages above matches what was intended to be sent. Applicants are advised to retain this receipt in the unlikely event that proof of this facsimile transmission is necessary. Applicants are also advised to use the certificate of facsimile transmission procedures set forth in 37 CFR 1.8(a) and (b), 37 CFR 1.6(f). Trademark Applicants, also see the Trademark Manual of Examining Procedure (TMEP) section 306 et seq.

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Cover
Page
=====>

01/06/2005 10:51 2155405818		HOWSON AND HOWSON		PAGE 01/14	
<p>Approved for use through 07/31/2006, OMB 0661-0031 U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE</p> <p>Under the Patent and Trademark Act of 1995, an applicant is required to respond to a collection of information unless it displays a valid OMB control number.</p>					
TRANSMITTAL FORM		<p>Application Number: 08231.047 Filing Date: May 3, 2001 First Named Inventor: Blaszczak-Thudn et al. Art Unit: 1633 Examiner Name: J. Anderson Attorney Contact Number: WATSONUSA</p> <p>Total Number of Pages in This Submission: 14</p>			
ENCLOSURES (Check all that apply)					
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment/Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavit/Declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Reply to Missing Parts/Incomplete Application <input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53		<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation <input type="checkbox"/> Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) <input type="checkbox"/> Landscape Table on CD <input type="checkbox"/> Remarks Customer No. 00270		<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Prioritary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) (please identify below): 3 pp. Second Request for Correction of Filing Receipt with 5 Exhibits	
SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT					
Firm Name: HOWSON AND HOWSON		Signature: Mary E. Bak			
Printed name: Mary E. Bak		Date: January 6, 2005			
Reg. No. 31,215					
CERTIFICATE OF TRANSMISSION/MAILING					
I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.					
Signature: Kelly A. Karsa		Date: January 6, 2005			
Typed or printed name: Kelly A. Karsa					
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PAGE 1/14 * RCVD AT 1/6/2005 10:52:37 AM [Eastern Standard Time] * SVR:USPTO-EFRCF-20 * DNS:7469195 * CSID:2155405818 * DURATION (mm-ss):03:50					

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TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	09/831,047	
	Filing Date	May 3, 2001	
	First Named Inventor	Blaszczyk-Thurin et al.	
	Art Unit	1653	
	Examiner Name	S. Snedden	
Total Number of Pages in This Submission	14	Attorney Docket Number	WST93AUSA

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment/Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Reply to Missing Parts/Incomplete Application <input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation <input type="checkbox"/> Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____ <input type="checkbox"/> Landscape Table on CD	<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) (please identify below): 3 pp. Second Request for Correction of Filing Receipt with 5 Exhibits
Remarks <div style="text-align: center;">Customer No. 00270</div>		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	HOWSON AND HOWSON		
Signature	<i>Mary E. Bak</i>		
Printed name	Mary E. Bak		
Date	January 6, 2005	Reg. No.	31,215

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Signature	<i>Kelly A. Karstaedt</i>		
Typed or printed name	Kelly A. Karstaedt	Date	January 6, 2005

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APR 20 2006

WST93AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/831,047

Confirmation No.: 8220

Applicant : Blaszczyk-Thurin et al.

Filed : May 3, 2001

TC/A.U. : 1653

URGENT!!

Examiner : S. Snedden

Customer No. : 00270

Title : COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

LETTER

Sir:

This letter is being filed to resubmit a Request for Correction of Filing Receipt, which was timely filed at the USPTO on May 9, 2002 to correct errors in the number of Independent Claims and the listing of Applicant(s). This paper is being filed after receipt of the Notice of Allowance dated December 27, 2004, but before payment of the issue fee due March 27, 2005.

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this date: January 6, 2005
Signature Kelly A. Karstaedt
Name Kelly A. Karstaedt

APR 20 2006

Enclosed as support for the timely filing of the Request for Correction of Filing Receipt are the following:

- (i) a two (2) page copy of the Request for Correction of Filing Receipt dated May 9, 2002 with accompanying transmittal papers (Exhibit AA);
- (ii) a one (1) page copy of a Postcard stamped by the USPTO indicating receipt of item (i) on May 22, 2002 by the USPTO (Exhibit BB);
- (v) a one (1) page copy of the Filing Receipt forwarded with the Request for Correction of Filing Receipt noting the errors in the number of independent claims and Applicant(s) (Exhibit CC);
- (vi) a two (2) page copy of the Transmittal Letter, which was filed with the application on May 3, 2001, and correctly recites the total number of independent claims as "10" (Exhibit DD); and
- (vi) a two (2) page copy of the Declaration, which was filed with the application on May 3, 2001, and correctly states the inventorship for this application as "Magdalena Blaszczyk-Thurin, Philadelphia, PA; Thomas Kieber-Emmons, Newtown Square, PA", and was forwarded with the Request for Correction of Filing Receipt (Exhibit EE).

Applicants respectfully assert that the above-indicated documents were timely filed at the United States Patent and Trademark Office in order to correct the following two errors in the Filing Receipt:

- Error 1: the number of Independent Claims should be 10, not 41; and
- Error 2: the listing of Applicant(s) should include Thomas Kieber-Emmons, Newtown Square, PA, so that the listing of Applicant(s) is correctly noted on the patent.

Applicants request correction of these two items.

The Director is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees to our Deposit Account Number 08-3040.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for Applicants

By Mary E. Bak
Mary E. Bak
Registration No. 31,215
Spring House Corporate
Center, Box 457
Spring House, PA 19477
Telephone: (215) 540-9200
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Kelly A. Karstardt	(Depositor's name)
Kelly A. Karstardt	(Signature)
January 25, 2005	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/831,047	05/03/2001	Magdalena Blaszczyk-Thurin	WST93AUSA	8220

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	\$700	\$0	\$700	03/28/2005

EXAMINER	ART UNIT	CLASS-SUBCLASS
SNEDDEN, SHERIDAN	1653	514-014000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.

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1 HOWSON AND HOWSON

2

3

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(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

The Wistar Institute of Anatomy and Biology

Philadelphia, Pennsylvania

The Trustees of the University of Pennsylvania

Philadelphia, Pennsylvania

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

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5. Change in Entity Status (from status indicated above)

☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature Mary E. BakDate Jan. 25, 2005Typed or printed name Mary E. BakRegistration No. 31,215

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Total Number of Pages in This Submission

2

Application Number

09/831,047

Filing Date

May 3, 2001

First Named Inventor

Blaszczyk-Thurin et al.

Art Unit

1653

Examiner Name

S. Snedden

Attorney Docket Number

WST93AUSA

ENCLOSURES (Check all that apply)

- ☐ Fee Transmittal Form
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☐ After Final
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Firm Name

HOWSON AND HOWSON

Signature

Mary E. Bak

Printed name

Mary E. Bak

Date

Jan 25, 2005

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Kelly A. Karstaedt

Date

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APR 20 2006

vivo. Indeed, SA-Lea specific monoclonal antibodies (MAbs) were inhibitory for adhesion of colon carcinoma cells to human umbilical cord vein endothelial cells (HUVEC).

In vivo studies have provided further evidence of the potential importance of the carbohydrate ligand/E-selectin interaction in tumor metastasis [Brodt *et al.*, 1997, *Int. J. Cancer* 71:612-619; Mannori *et al.*, 1997, *Am. J. Pathol.* 151:233-243 (Mannori II); Biancone *et al.*, 1996, *J. Exp. Med.* 183:581-587].

Alternatively, some carcinoma cells do not express these carbohydrate determinants (i.e., SA-LeX and SA-Le^a) and yet they can attach to EC prior to activation. Further, this adhesion is not augmented by cytokine treatment, suggesting E-selectin-independent adhesion [Iwai *et al.*, 1993, *Int. J. Cancer* 54:972-977; Tozeren *et al.*, 1995, *Int. J. Cancer* 60:426-431; Miyake *et al.*, 1992, *New Eng. J. Med.* 327:14-18; Garrigues *et al.*, 1992, *J. Cell. Biol.* 125:129-142].

Studies have also demonstrated the role of oligosaccharides in inflammatory responses. Neutrophil extravasation is enabled by a multistep process initiated by the selectin family [Kansas, 1996, *Blood* 88: 3259-3287]. Neutrophil-endothelial cell interaction mediated via the selectins in the context of vascular shear flow, are characterized by transient tethering of the neutrophils, followed by rolling of the neutrophil along the endothelial surface of the vessel wall. Studies *in vivo* and *in vitro* indicate that selectin-dependent neutrophil rolling is essential to subsequent events in the transmigration process. Neutrophils are exposed to endothelial cell derived IL-8, platelet-activating factor and other neutrophil-activating molecules [Lowe, 1997, In: The selectins: Inhibitors of leukocyte endothelial adhesion, pp. 143-177, Vestweber, ed., Harwood Academic Publishers, Reading, UK], which in turn promote activation of neutrophil P2 integrins, leading to integrin-dependent firm adhesion to the integrin receptor ICAM-1, and finally to neutrophil extravasation, possibly via homophilic interaction of platelet/endothelial cell adhesion molecule 1.

The expression of ligands for selectins, particularly E-selectin, by both neutrophils and carcinoma cells raises the possibility that metastases are equivalent to the inflammatory process in which tumor cells, particularly carcinoma cells, use the

MAB were incubated with increasing amounts of peptides and binding of free antibody to carbohydrate SA-Le^a was measured by enzyme linked immunosorbent assay.

Results show competitive inhibition of MAB binding to solid phase SA-Le^a

polyacrylamide matrix (SA-Le^a-PAA) by 12-mer peptides DLWDWVVGKPAG (■)

5 [SEQ ID NO: 1] and DLWDFVVGKPAG (▲) [SEQ ID NO: 63] with respect to the MAB binding without peptide (100% of binding) and a negative control unrelated peptide (○).

Fig. 4 is a circular dichroism (CD) spectra comparing dodecapeptides DLWDWVVGKPAG (solid line) [SEQ ID NO: 1] and DLWDFVVGKPAG (---)

10 [SEQ ID NO:63]. The spectra were recorded at 0.51 mg/ml for both peptides.

Fig. 5 is a graph illustrating the inhibition of lung experimental metastases with peptide DLWDFVVGKPAG [SEQ ID NO: 63]. Tumor cells were admixed with the specific or unrelated peptide solution (1 mg per mouse) and animals were inoculated with 1×10^5 B16F10FtIII tumor cells in 200 μ l volume of PBS via tail vein. Results are from 4 experiments (5 mice in each group) are shown. Each dot represents enumerated tumor nodules in one lung in experimental group of C57Bl/6 mice treated with the peptide (*panel B*), control group of C57Bl/6 mice treated with unrelated peptide (*panel A*) and E-selectin knock-out (KO) mice of C57Bl/6 background (*panel C*). Statistical analysis using a nonparametric unpaired *t* test gave a two-tailed *p* values <0.008 and 0.009 for animals treated with peptide and E-selectin KO, respectively, as compared to control group. The horizontal bars represent median values and vertical bars denote standard deviation.

Detailed Description of the Invention

The invention is based on the discovery that peptido-mimetics of complex carbohydrate structures block adhesion of tumor cells and leukocytes to endothelial cells. The production of small peptide molecules which mimic complex carbohydrate structures are useful for blocking carbohydrate ligand-cell adhesion molecule interactions involved in metastasis, angiogenesis, and inflammatory responses. Thus, these peptido-mimetics are useful as anti-adhesion therapeutics. The evaluation of

VGNGVLMGRRG [SEQ ID NO:90], RVLSPESYLGPA [SEQ ID NO:92],
GNCRYIGLRQFG [SEQ ID NO:93], DIRVEPGGGYTH [SEQ ID NO:94],
APIHTYTGRARG [SEQ ID NO:96], and RHTCVRSCGHDR [SEQ ID NO:97].

Similarly, exemplary peptido-mimetics of SA-Le^a include, without
5 limitation, VGIWSVVSEGSR [SEQ ID NO:102], RCSVGVPFTMES [SEQ ID
NO:103], QDGVWEHVLEGG, [SEQ ID NO:104], DLWDWVVGKPAG [SEQ ID
NO:1], VELSGRGGLCTW [SEQ ID NO:105], VIGAASHDEDVD [SEQ ID
NO:106], TIEPVLAEMFMG [SEQ ID NO:107], DKETFELGLFDR [SEQ ID
NO:108], FSGVRGVYESRT [SEQ ID NO:109], PDDAPMHSTRVE [SEQ ID
10 NO:110], STGLMVDFLEPG [SEQ ID NO: 91], AKTFGLEHGCEA [SEQ ID NO:
95], GGTVEVWSIKGG [SEQ ID NO: 115], DHFSQAGSSNHH [SEQ ID NO:
116]; DDPVTPVIDFGK [SEQ ID NO: 117], and RDGLIDFVVAGT [SEQ ID NO:
118].

As described in the examples below, families of mimics of carcinoma-
15 associated antigens that represent SA-Le^a, in particular, were identified from a
combinatorial peptide library using MAb NS19-9 specific for this carbohydrate
structure. One of the peptides, DLWDWVVGKPAG [SEQ ID NO: 1], was selected
that specifically competes for binding of MAb for SA-Le^a. This peptide displays an
ability to partially inhibit neutrophil recruitment in an acute inflammation model *in*
20 *vivo*. As described below, this peptide was analyzed by systematic amino acid
replacements to identify optimal conformationally stabilized SA-Le^a mimics with
higher affinity using a solid phase peptide array library. Comparison of signal
intensities revealed significant differences in MAb binding as a result of substitutions,
in particular at the N-terminus. Substitution analysis allowed for delineation of key
25 residues that were sensitive to replacement. MAb NS19-9 discriminated against
multiple amino acid substitutions affecting its recognition. Specific residues within
this peptide were identified that may contribute to the mimicry of carbohydrate
structure by the peptide.

On the other hand MAb NS19-9 could tolerate replacement of the lead
30 peptide sequence by a variety of amino acids. These substitutions did not abrogate

databases for small molecular compounds include Cambridge Structural Database, Fine Chemical Database, and CONCORD database [for a review see Rusinko, A., Chem. Des. Auto. News, 8:44-47 (1993)].

5 Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound. Assembly may proceed by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure of the peptido-mimetic of this invention. Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include the CAVEAT program [P. A. Bartlett
10 et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", in Molecular Recognition in Chemical and Biological Problems", Special Pub., Royal Chem. Soc. 78, pp. 182-196 (1989)], which is available from the University of California, Berkeley, CA; 3D Database systems such as MACCS-3D database (MDL Information Systems, San Leandro, CA) [see, e.g., Y.
15 C. Martin, "3D Database Searching in Drug Design", J. Med. Chem., 35:2145-2154 (1992)]; and the HOOK program, available from Molecular Simulations, Burlington, MA.

Compounds that mimic a peptide of this invention or a ligand of the peptides may be designed as a whole or "*de novo*" using either an empty active site or
20 optionally including some portion(s) of a known ligand(s). Suitable methods describing such methods include the LUDI program [H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", J. Comp. Aid. Molec. Design, 6:61-78 (1992)], available from Biosym Technologies, San Diego, CA; the LEGEND program [Y. Nishibata and A. Itai, Tetrahedron,
25 47:8985 (1991)], available from Molecular Simulations, Burlington, MA; and the LeapFrog program, available from Tripos Associates, St. Louis, MO. Other molecular modelling techniques may also be employed in accordance with this invention. See, e.g., N. C. Cohen et al, "Molecular Modeling Software and Methods for Medicinal Chemistry", J. Med. Chem., 33:883-894 (1990). See also, M. A. Navia
30 and M. A. Murcko, "The Use of Structural Information in Drug Design", Current

Opinions in Structural Biology, 2:202-210 (1992). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the peptide of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, e.g., P.S. Farmer, Drug Design, Ariens, E.J., ed., Vol. 10, pp 119-143 (Academic Press, New York, 1980); U.S. Patent No. 5,331,573; U.S. Patent No. 5,500,807; C. Verlinde, Structure, 2:577-587 (1994); and I. D. Kuntz, Science, 257:1078-1082 (1992). The model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Thus, using these computer evaluation systems, a large number of compounds may be quickly and easily examined and expensive and lengthy biochemical testing avoided. Moreover, the need for actual syntheses of many compounds is effectively eliminated. Once identified by the modelling techniques, the proposed "new antibacterial" compound may be tested for bioactivity using standard techniques, such as the assays of the examples below.

The invention is further described in detail by reference to the following, experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLE 1: SCREENING OF A RANDOM PEPTIDE LIBRARY

To develop novel molecules to inhibit the adhesion of human adenocarcinoma cells to EC and, ultimately, to inhibit metastasis *in vivo*, peptides were derived from a 12-mer random peptide library. A diverse library of random dodecapeptides, displayed as flagellin-thioredoxin fusion proteins (FLITRX) on the surfaces of *E. coli* bacterial cells, was obtained from Invitrogen (Carlsbad, CA) [LaVallie *et al.*, 1993, *Bio/Technology*, 11:187-193]. This library enables efficient isolation of bacteria displaying peptides with affinity to immobilized antibodies or to other binding proteins.

very good. This peptide competes with LeY for BR55-2 binding. These data further demonstrate another method to develop peptide mimotopes that are specific. This method may be extended to identify motifs mimicking SA-LeX and SA-Le^a using the crystal structure of the lectin domain of E-selectin in optimizing respective mimotopes.

5 EXAMPLE 6: IDENTIFICATION OF SEQUENCES CRITICAL FOR MAB
 BINDING AND AN SA-Le^a MIMIC WITH HIGHER ANTIBODY BINDING
 AFFINITY

To analyze amino acid residues that are critical for NS19-9 recognition, an array library of 163 unique peptides was generated by systematic amino acid
10 replacement in which each position of the starting peptide DLWDWVVGKPAG [SEQ ID NO: 1] was replaced by other L-amino acids. In addition, peptides were synthesized with simultaneous incorporation of multiple amino acids or with truncation of specific regions.

The peptide array of 163 unique peptides was generated by substituting all
15 amino acids for each individual amino acid in lead peptide (DLWDWVVGKPAG [SEQ ID NO: 1] identified by combinatorial library panning with MAb NS19-9. An array of synthetic 12-mer peptides was synthesized using 90 x 130 mm polyethylene glycol-modified cellulose membrane functionalized with approximately 4 nmole/mm² amino groups, manufactured by Abimed (Lagenfeld, Germany). Standard Fmoc chemistry was used according to the manufacturer's instructions [Frank, R., 1992, *Tetrahedron* 48, 9217-9332]. The protected and activated amino acids were spotted
20 using an Abimed ASP 422 robotic arm. All washing, dyeing and deprotection steps were done manually. The activated C-terminal amino acids were spotted leaving 10 mm space in each direction, at the concentration of 0.5 M in N-methyl pyrrolidone. A volume of 0.5 ml provides spot of 7-8 mm in diameter. Activation of the amino acids
25 with dicyclohexyl-carbodiimide and N-hydroxy-benzotriazole was done 30 minutes before spotting. After each coupling cycle, the paper was washed with 12% acetic anhydride dissolved in N,N'-dimethylformamide (DMF) twice for a total of 10 min to endcap all unreacted amino groups. Repetitive removal of the Fmoc groups was

Table 4(cont'd)

Peptide Sequence	SEQ ID NO	Peptide Sequence	SEQ ID NO
EIHDWVVGKPAG	49	WDWVVGKPAG	56
DLWEHL	50	DWVVGKPAG	57
LDDDWVVGKPAG	51	WVVGKPAG	58
EIHEWVVGKPAG	52	VVGKPAG	59
DLWDHLLA	53	VGKPAG	60
LDDLWVVGKPAG	54	GKPAG	61
EIHEHLVGKPAG	55	KPAG	62

Comparison of signal intensities on the peptide array revealed that some substitutions led to enhanced NS19-9 binding allowing for identification of several peptides with increased binding affinity to the antibody (Table 5). Improvement of peptide binding was achieved mainly by substitution of residues 5 to 12 within the lead peptide, whereas no amino acid exchange at N-terminus (residues 1-4) led to the increased binding. The replacement of residues with amino acids containing polar groups such as Glu and Asp showed clearly an enhancing effect at the C-terminus but not the N-terminus.

Array analysis failed to reveal significant differences in the binding intensities between the peptides substituted at different positions, suggesting that single substitution at any position in this region with carboxyl groups can enhance the interaction with the MAb binding site. In addition, substitutions with Ile, Ala and Ser also improved MAb binding. Similarly, the simultaneous replacement of several residues with clusters of amino acids upstream from position 6 demonstrated enhanced binding. The highest intensity signal was, however, observed with peptide DLWDFVVGKPAG [SEQ ID NO:63] containing a single substitution at position 5 with Phe.

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Table 5 lists the amino acid substitutions within peptide DLWDWVVGKPAG [SEQ ID NO: 1] that increase the binding of MAb NS19-9 using the peptide array.

Table 5

Peptide Sequence	SEQ ID NO	Peptide Sequence	SEQ ID NO
DLWDFVVGKPAG	63	DLWDWVVGKPDG	73
DLWDWVIGKPAG	64	DLWDWVVGKPAD	74
DLWDWVVAKPAG	65	DLWDWVKEKPAG	75
DLWDWVVS KPAG	66	DLWDWVLAKPAG	76
DLWDWVVEKPAG	67	DLWDWVVGEDAG	77
DLWDWVVDPKAG	68	DLWDWVVGKPEK	78
DLWDWVVGEPAG	69	DLWDWVKEEPAG	79
DLWDWVVGDPAG	70	DLWDWVVGKDEK	80
DLWDWVVGKEAG	71	DLWDWVVGEDEK	81
DLWDWVVGKDAG	72	DLWDWVKEEDEK	82

A distinct pattern of substitutions that led to increased or abolished signal intensities with respect to the C- and N-terminus suggests that the region close to the N-terminus might contribute to the specificity of the interaction with NS19-9. Amino acids close to the C-terminus appear to add significantly to the affinity of ligand binding.

EXAMPLE 7: INHIBITION OF BINDING OF MAb TO THE CARBOHYDRATE WITH SYNTHETIC PEPTIDES MIMICKING SA-Le^a STRUCTURE

DLWDWVVGKPAG [SEQ ID NO:1] and DLWDFVVGKPAG [SEQ ID NO:63] were chemically synthesized and tested for their ability to compete to immobilized synthetic SA-Le^a-PAA neoglycoprotein. To determine whether peptide SEQ ID NO: 1 and peptide SEQ ID NO: 63 are true mimics of SA-Le^a, dose-response experiments were carried out in order to determine the concentration of peptides

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claims are intended to be construed to include all such embodiments and equivalent variations.

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PTO/SB/21 (09-04)

Approved for use through 07/31/2006. OMB 0651-0031

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TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	09/831,047	
	Filing Date	May 3, 2001	
	First Named Inventor	Blaszczyk-Thurin	
	Art Unit	1653	
	Examiner Name	S. Snedden	
Total Number of Pages in This Submission	7	Attorney Docket Number	WST93AUSA

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input checked="" type="checkbox"/> Amendment/Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Reply to Missing Parts/ Incomplete Application <input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____ <input type="checkbox"/> Landscape Table on CD	<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input type="checkbox"/> Other Enclosure(s) (please identify below):
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/831,047 Confirmation No.: 8220
Applicant : Blaszczyk-Thurin
Filed : May 3, 2001
TC/A.U. : 1653
Examiner : S. Snedden
Customer No. : 00270
Title : COMPOSITIONS AND METHODS FOR TREATMENT OF
CANCER

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE AND AMENDMENT

Sir:

This paper is in timely response to the Office Action dated September 13, 2004.
Please enter the following remarks and amendments.

Amendments to the Claims are reflected in the listing of claims which begins on
page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

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APR 20 2005

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-5 (Canceled).

6(Currently Amended). The A peptide or polypeptide according to claim 3 which is selected from the group consisting of: ASAVNLYIPTQE SEQ ID NO:84, VYLAPGRISRDI SEQ ID NO:85, VYLAPGRFSRDI SEQ ID NO:86, CTSHWGVLSQRR SEQ ID NO:87, RVLSPESYLGPS SEQ ID NO:88, VGNGVLMGRRG SEQ ID NO:90, RVLSPESYLGPA SEQ ID NO:92, GNCRYIGLRQFG SEQ ID NO:93, DIRVEPGGGYTH SEQ ID NO:94, APIHTYTGRARG SEQ ID NO:96, and RHTCVRSCGHDR SEQ ID NO:97 which binds to a selectin and blocks binding between said selectin and a Lewis SA-Le^a or SA-LeX antigen.

Claim 7(Canceled).

8(Currently Amended). The A peptide or polypeptide according to claim 3, wherein said Lewis antigen is SA-Le^a and said peptide or polypeptide is selected from the group consisting of VGIWSVVSEGSR SEQ ID NO:102, RCSVGVPFTMES SEQ ID NO:103, QDGVWEHVLEGG, SEQ ID NO:104, DLWDWVVGKPAG SEQ ID NO:1, VELSGRGGLCTW SEQ ID NO:105, VIGAASHDEDVD SEQ ID NO:106, TIEPVLAEMFMG SEQ ID NO:107, DKETFELGLFDR SEQ ID NO:108, FSGVRGVYESRT SEQ ID NO:109, PDDAPMHSTRVE SEQ ID NO:110, STGLMVDFLEPG SEQ ID NO:91, AKTFGLEHGCEA SEQ ID NO:95,